Journal of Experimental Botany, Vol. 59, No. 4, pp. 973–979, 2008
doi:10.1093/jxb/ern023 Advance Access publication 2 March, 2008
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Silencing polygalacturonase expression inhibits tomato petiole abscission

Cai-Zhong Jiang[2], Feng Lu[1], Wachiraya Imsabai[1], Shimon Meir[3] and Michael S. Reid[1,]*

[1] *Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA*
[2] *Crops Pathology and Genetics Research Unit, USDA, ARS, One Shields Avenue, Davis, CA 95616, USA*
[3] *Department of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel*

Received 6 November 2007; Revised 11 January 2008; Accepted 16 January 2008

Abstract

Virus-induced gene silencing (VIGS) was used as a tool for functional analysis of cell wall-associated genes that have been suggested to be involved in leaf abscission. Tobacco rattle virus is an effective vector for VIGS in tomato (*Lycopersicon esculentum*). Silencing was more efficient when the plants were grown at 22 °C than when they were grown at 20 °C or 25 °C. The photobleaching phenotype resulting from silencing phytoene desaturase varied, depending on cultivar, from barely visible to photobleaching of entire leaves. To study the function of abscission-related genes, a purple transgenic tomato line constitutively expressing the maize anthocyanin regulatory gene, *Leaf colour* (*Lc*) was used. Silencing *Lc* expression in this line resulted in reduced anthocyanin production (reversing the colour from purple to green), thus providing a convenient silencing 'reporter'. Silencing tomato abscission-related polygalacturonases (TAPGs), using a *TAPG1* fragment, delayed abscission and increased break strength of the abscission zone in explants treated with 1 μl l^{-1} ethylene. The abundance of *TAPG1* transcripts in the green (silenced) abscission zone tissues was <1% of that in the purple (non-silenced) controls. As a highly homologous region was used for all five polygalacturonases it is assumed that the effect of delayed abscission is the result of silencing all the genes in this family. By contrast, silencing abscission-related expansins (LeEXP11 and LeEXP12) and endoglucanases (LeCEL1 and LeCEL2) had no discernible effect on break strength, even when the two endoglucanase genes were silenced concurrently. Simultaneous silencing of TAPG and LeCEL1 was no more effective than silencing TAPG alone. The data demonstrate the importance of TAPGs in the abscission of leaf petioles.

Key words: Abscission, cellulase, endoglucanase, expansin, *Solanum lycopersicum*, polygalacturonase, virus-induced gene silencing.

Introduction

Abscission is defined as a part of plant development in which subtended organs (i.e. leaves, flowers, fruits, and even branches) separate from the plant. Many researchers have provided evidence supporting the hypothesis that the primary events in the separation zone include disassembly of cell wall components (Roberts *et al.*, 2002). It has been demonstrated that transcript abundance and activities of several cell wall-modifying proteins, including endoglucanases (Tucker and Milligan, 1991; Tucker *et al.*, 1991; del Campillo and Bennett, 1996; Mishra *et al.*, 2008), polygalacturonases (Kalaitzis *et al.*, 1995, 1997; Gonzalez-Carranza *et al.*, 2002, 2007), expansins (Cho and Cosgrove, 2000; Belfield *et al.*, 2005), and xyloglucan endotransglucosylase/hydrolases (Campbell and Braam, 1999) increase during the abscission process. In tomato plants transformed with a construct driving antisense expression of the endoglucanase genes, *LeCEL*1 (Lashbrook *et al.*, 1998) and *LeCEL*2 (Brummell *et al.*, 1999*a*), flower abscission, but not fruit softening, was somewhat reduced, implying a role for endoglucanases in floral abscission. These researchers did not report the effect of endoglucanase down-regulation on leaf abscission.

Cho and Cosgrove (2000) demonstrated that pedicel abscission in *Arabidopsis* was enhanced by overexpression and reduced by antisense suppression of *AtEXP10*, an

* To whom correspondence should be addressed. E-mail: msreid@ucdavis.edu

expansin. Tucker and his colleagues (Kalaitzis *et al*., 1995, 1997; Hong *et al*., 2000) have cloned genes encoding tomato polygalacturonases (TAPGs), and have demonstrated expression patterns that are suggestive of a potential role in abscission of leaves and flowers. However, there has been no definitive demonstration of a role for a TAPG in tomato leaf or floral abscission. Disruption of an abscission-related polygalacturonase (PG) in an *Arabidopsis* T-DNA insertional mutant delayed the time-course of floral organ loss but did not prevent shedding from taking place (Gonzalez-Carranza *et al*., 2007).

Virus-induced gene silencing (VIGS) is a method of gene silencing that has been successfully used in functional analysis of genes associated with development and senescence (Chen *et al*., 2004). Liu *et al*. (2002) and Fu *et al*. (2005) demonstrated that VIGS using tobacco rattle virus (TRV) vectors could be used to silence expression of phytoene desaturase (PDS) in tomato leaves and fruits. In petunia, it was found that the phenotype of plants in which genes were silenced was highly dependent on the cultivar and growing conditions (Chen *et al*., 2004). Here a similar variation in the response of tomatoes is reported. As in other VIGS systems, silencing occurs in a mosaic, presumably reflecting non-uniform systemic movement of the virus, so using this technique for testing the effect of silencing one or more genes requires a visible silencing reporter. Although PDS gives a clear phenotype in tomato (Liu *et al*., 2002; Fu *et al*., 2005, 2006), it is not a satisfactory reporter, because the effect of silencing target genes may be confounded by the physiological effects of the leaf photobleaching and the growth abnormalities that result from silencing PDS.

In purple flowered petunias, silencing of chalcone synthase (CHS) and the resulting loss of purple pigmentation, was used as a reporter for the silencing of floral genes (Chen *et al*., 2004). Goldsbrough *et al*. (1996) created a dark purple tomato line by transforming tomatoes with *Lc* (Leaf colour), an anthocyanin regulatory gene from maize. By inserting an *Lc* fragment and fragments of genes encoding cell wall-modifying proteins into the VIGS vector, it was possible to use VIGS to conduct a functional analysis of the effect of silencing these genes on leaf abscission in seedlings of this tomato line. Green tissues in the normally purple plants indicated where silencing of Lc (and of the cell wall-modifying proteins) were taking place.

Materials and methods

Plant material

A range of tomato cultivars was grown in order to evaluate cultivar and temperature effects on the efficiency of the TRV silencing system, using PDS as a reporter. The seeds were germinated at 22 °C in moistened rolled paper towels, and the plants were grown in controlled temperatures (20, 22, or 25 °C) and continuous light from cool-white fluorescent lamps. For functional analysis of cell wall-modifying proteins, a dark purple tomato line obtained from John Yoder (Plant Sciences, UC Davis) was used. Plants of the 'New Yorker' cultivar were transformed with a maize anthocyanin control element (Lc) driven by the constitutive 35S promoter (Goldsbrough *et al*., 1996). These plants were grown in growth chambers under 16 h light/8 h dark cycles with a day/night temperature regime of 22/19 °C.

VIGS vector

Gene silencing was conducted using a TRV vector kindly provided by Dinesh Kumar (Yale University). The pTRV1 and pTRV2 vectors for VIGS have previously been described in detail (Liu *et al*., 2002; Chen *et al*., 2004). For testing cultivar variation, the effect of silencing PDS was examined. For testing the role of cell wall-modifying enzymes in abscission, a fragment of the *Lc* gene (203 bp), together with fragments (150–400 bp) of genes of interest were inserted. The resulting constructs were transformed into *Agrobacterium tumefaciens GV3101*.

PDS *construct*

A 138 bp fragment of the *PDS* gene was PCR-amplified from tomato cDNA using primers 5′-CAGTGCTTCTTGATCGCTTTGA-3′ and 5′-TCTGACTTGGCCACCTTTTGA-3′. The resulting product was cloned into pTRV2 to form pTRV2–*PDS*.

Lc *construct*

A 203 bp fragment of the *Lc* gene was PCR-amplified from tomato cDNA generated from Lc overexpressed transgenic tomato lines using primers 5′-AGCGACGAGAGAAGCTCAAC-3′ and 5′-GGAGGGCCTTGTTATTAGCC-3′. The resulting product was cloned into pTRV2 to form pTRV2–*Lc*.

Cell wall-modifying enzyme constructs

Fragments of genes encoding cell wall-modifying proteins were PCR-amplified from tomato cDNA using the primers shown in Table 1. The resulting products were sequenced to confirm their identity, and then cloned into pTRV2-*Lc* to generate pTRV *gene fragment*/*Lc* constructs.

Infection

Young seedlings were infected with a mixed culture of *A. tumefaciens* bearing the RNA1 and modified RNA2 of TRV.

Table 1. *List of primers used for isolation of PG (TAPG1/2), endoglucanases (LeCEL1 and 2), and expansins (LeEXP11 and 12)*

Gene	Forward primer	Reverse primer	Product size
TAPG1/2	CACAGATGGAATTCATGTAA	CCAGAACCCTGATGAGGACA	384
LeCEL1	CTTGAGGCAGCTGGAATAGG	GCACCGACATGTGTGTTAGG	281
LeCEL2	AAACACATTGCCAAACGTCA	CTGTTAAATTTGGCCCTCCA	241
LeEXP11	CTTGTGGCTATGGGAACTTGTACT	AACAATGCCACCTCTGTAGATACC	299
LeEXP12	GTCCCTGTTGCTTATAGAAGGGTA	GAGCACCAGTATATGTCTGTCCAA	316

Seedlings, still in the rolled paper towels that were used for germination, were placed in a capped 50 ml syringe with the mixed *A. tumefaciens* culture and pressurized by depressing the plunger for 3 min. The infected seedlings were planted in soil, maintained in high humidity for several days, and then grown under incandescent lamps in a Conviron (PGC20; Controlled Environments Limited, Winnipeg, Manitoba, Canada) growth chamber at 22 °C day/19 °C night in a 16 h photoperiod.

Abscission assay

Explants were prepared from young plants with four or five expanded leaves. Green plants (showing silencing of *Lc* and therefore of the gene of interest) were used to test the effect of silencing. Purple plants served as controls. Explants comprising the middle (2nd or 3rd) petiole and the subtending proximal and distal internodes were used for abscission testing (Fig. 2B). The explants were placed with their bases in 1.5 ml Eppendorf tubes containing 1 ml water, and were then exposed to 1 μl l^{-1} ethylene in a flowing air stream (35 l h^{-1}) at 20 °C. At intervals, explants were removed from the ethylene treatment tank, and the force required to remove the petiole from the stem was determined using a Stable Micro System Texture Analyser (Texture Technologies Corp, Scarsdale, NY, USA). The explant was placed in a saddle attached to the head of the analyser, and the end of the petiole was held with a small vice attached to the base of the instrument. The force (N) required to separate the petiole from the axis was then determined by raising the analyser head at a speed of 1 mm s^{-1}.

Determination of transcript abundance

After the abscission assay, abscission zone tissue (1 mm from each side of the separation layer) was dissected, frozen in liquid nitrogen, and stored at –80 °C until needed. Total RNA was extracted from the tissue samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and treated with RNase-free DNase I (Promega, Madison, WI, USA) to remove any contaminating genomic DNA. First-strand cDNA, synthesized as described previously (Chen *et al.*, 2004), was used as a template to determine the abundance of *Lc* and other gene transcripts, using semi-quantitative PCR followed by real-time PCR (ABI7300; Applied Biosystem, Foster City, CA, USA) with SYBR Green reagent. The PCR primers for amplifying VIGS target gene transcripts were designed outside the region targeted for gene silencing to avoid amplification from the TRV/VIGS genome. Primers used for determining *TAPG* transcript abundance were 5′-TATGGATTGAAGGCATTGC-3′ and 5′-TGTAGCTGATGTTC-CATGTAT-3′. The abundance of *18S* rRNA, determined using the amplification primers 5′-CATGGCCGTTCTTAGTTGGTGGAG-3′ and 5′-AAGAAGCTGGCCGCGAAGGGATAC-3′, served as an internal control. Data were analysed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and are presented as the relative levels of gene expression.

Results

Tomato cultivars vary dramatically in their response to VIGS

The timing, efficiency, and phenotypic response to silencing of PDS (which was used in initial studies to optimize silencing efficiency) varied substantially among species and cultivars (Table 2). The time from infection to the appearance of first symptoms ranged from 8 d for ‘Amana orange’ to 14 d for ‘Celebrity’. Infection efficiency ranged from 50% (‘Red Top VF’) to 100% (19 out of the 25 cultivars tested), and the phenotype ranged from barely noticeable to almost complete photobleaching.

Temperature has a strong effect on silencing

On average, over all cultivars tested, and under continuous illumination and temperature, the speed of the response, the efficiency of infection, and the clarity of the phenotype were optimal at 22 °C (Table 3), and this temperature was used in subsequent silencing experiments. Both at lower and higher temperatures, efficiency and strength of the phenotype was reduced, and the time from infection to obvious symptoms was extended.

Table 2. *Response of a range of tomato cultivars to infection at 22 °C with TRV containing a fragment of the PDS gene*

Photobleaching was evaluated when the phenotype had fully developed, using a hedonic scale: 0, no visible symptoms; 5, severe photobleaching. n/a = not available.

Cultivars	% Infection	Photobleaching score	Days to phenotype
Micro Tom	100	0.7	10
New Yorker	100	3.5	10
Pearson	100	0.8	n/a
Hacienda Calera	100	3.7	n/a
Red Top VF	50	4.5	n/a
VFNT (Cherry)	80	3.1	n/a
M-28	80	2.4	n/a
Florida 7547	100	3.3	n/a
Condine Red	100	3.0	n/a
King’s Choice Hybrid	100	2.0	11
Jubilee	100	0.9	12
Jelly Bean Hybrid	100	1.8	10
Oregon Spring	75	4.0	13
Black Krim	100	2.5	13
Beefsteak	100	3.5	10
Gardeners Delight	100	2.8	10
Celebrity	75	4.0	14
Amana Orange	75	1.2	8
Ace 55 VF	100	3.4	12
Marglobe	100	3.7	10
San Marzona	100	2.0	13
Lemon Boy	100	3.5	11
First Lady	100	3.8	11
Bradley Pink	100	4.8	n/a
Brandywine Red	100	1.0	12

Table 3. *Effects of growth temperature on the timing, efficiency, and phenotypic response to silencing of the PDS gene in tomato*

The values are means ±SD for all cultivars tested.

Growth temperature (°C)	% Plants with photobleaching	Photobleaching score (0–5)	Days to phenotype
20	86	1.6±1.0	14
22	94	2.8±1.2	10
25	70	1.5±0.8	14

Silencing Lc *results in a distinct silencing phenotype*

When 'New Yorker' tomato seedlings heterozygous for the *Lc* transgene were infected with a TRV construct containing an *Lc* fragment, a high percentage of the plants grew without the dark purple coloration normally seen in the uninfected and vector only plants (Fig. 1). In green (silenced) abscission zone tissues, the abundance of *Lc* transcripts was reduced to between 0.2% and 9% of that in purple (non-silenced) tissues (Table 4).

Silencing TAPG1 *delays abscission*

The mean force required to remove petioles from control explants fell steadily, commencing about 24 h after the start of C_2H_4 treatment. By 72 h, it was impossible to measure break strength in the Lc controls because many of the petioles were already separated or separating from the axis. In explants where *TAPG1* had been silenced there was a clear delay in the fall in break strength, and the force required to remove the petioles was higher than for the controls throughout the experiment (Fig. 2). In subsequent experiments evaluating the effect of silencing different genes on petiole abscission, break strength was measured 60 h after the start of the C_2H_4 treatment.

Other genes whose products have been suggested to be important during abscission, including *LeEXP11*, *LeEXP12*, *LeCEL1*, and *LeCEL2*, were silenced. In addition, tandem constructs were used to silence pairs of genes. These tandem constructs comprised fragments of *Lc*, *LeCEL,1*, and *LeCEL2*, or *Lc*, *TAPG1*, and *LeCEL1*. Apart from plants where *TAPG1* had been silenced, the force required for petiole abscission was very close to that shown by controls for all of the silenced genes (Fig. 3).

Fig. 1. Virus-induced silencing of *Lc* reduces anthocyanin accumulation. Leaves (A) and explants (B) from control (left, vector only) and *Lc*-silenced (right, vector containing a fragment of the *Lc* gene) can clearly be distinguished by the difference in their anthocyanin pigmentation.

Silencing *TAPG1* more than doubled the break strength of the abscission zone at 60 h. Break strength of petioles where *TAPG1* and *LeCEL1* were silenced in tandem was not significantly different from those where *TAPG1* was silenced alone.

Silencing reduces transcript abundance

For the genes tested, silencing resulted in a strong decrease in transcript abundance. In the *Lc*-silenced petiole abscission zones, *Lc* transcript abundance, measured using real-time PCR, was reduced to <10% of that in the plants infected with the empty vector (Table 4). When a *TAPG1* fragment was included in the vector, silencing of *TAPG1* was even more dramatic, with *TAPG1* transcripts being barely detectable in the abscission zones during the period of normal abscission (48–72 h) (Table 4).

Discussion

Our data demonstrate an important role for PG in petiole abscission. Over the years, numerous workers have reported correlations between changes in the expression of a range of cell wall-related enzymes and the events of abscission. For example, Clements and Atkins (2001) reported reduced activities of endoglucanasess and PG during ethylene exposure of a single-gene recessive mutant of lupin that does not abscise any organs. However, their study, while clearly demonstrating an association between loss of enzyme activity and the non-abscising condition did not prove that the two phenotypes were functionally related. Most functional studies have focused on endoglucanases, whose activity has been reported to change dramatically during the execution phase of abscission. There have been numerous studies (for a review, see Roberts *et al.*, 2002) showing increased activity of endoglucanases in leaf abscission zones. As long ago as 1991, Tucker *et al.* (1991) isolated and sequenced a cDNA encoding an endoglucanase that was strongly and specifically up-regulated in bean leaf abscission zones. Sequence comparison demonstrates that the protein sequence for this gene (AAC78504) is highly homologous (71% identity, 87% similarity) to that of LeCEL1 (U13054). In floral and fruit abscission, functional analysis using anti-sense suppression has demonstrated a role for LeCEL1 (Lashbrook *et al.*, 1998) and LeCEL2 (Brummell *et al.*, 1999*a*) endoglucanases. Although these researchers showed delayed abscission following partial silencing of endoglucanase genes, they concluded that LeCEL1 activity is insufficient to account for all floral abscission (Lashbrook *et al.*, 1998), and that LeCEL2 contributes to cell wall disassembly during tomato fruit abscission (Brummell *et al.*, 1999*a*). These results implicate the involvement of enzymes other than endoglucanase in abscission, but it is certainly surprising

Table 4. *Abundance of* TAPG1 *and* LC *transcripts in abscission zones excised from plants infected with the TRV vector only (control) or with the TRV containing fragments of the reporter gene* Lc *with* TAPG1

Explants were treated with 1 μl l^{-1} ethylene, and samples were made at intervals after the start of the treatment. ΔCT values represent the difference in cycle number from the 18S controls, and the percentage reduction in abundance is calculated from the ΔΔCT. n.d. indicates that no product was detected after 40 cycles.

	Hours in C_2H_4								
	48			60			72		
	Control	*TAPG1/Lc*		Control	*TAPG1/Lc*		Control	*TAPG1/Lc*	
	ΔCT	ΔCT	%	ΔCT	ΔCT	%	ΔCT	ΔCT	%
Lc	2	10.9	0.2	7	10.5	8.8	8.6	13.1	4.4
TAPG1	9.9	n.d.	n.d.	11.4	21.1	0.1	13.7	n.d	n.d.

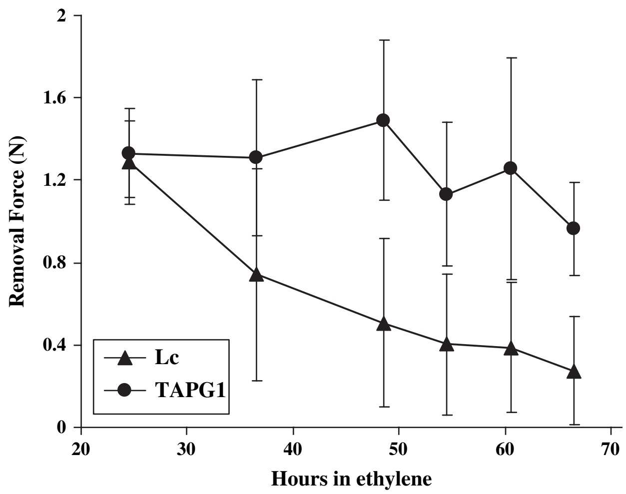

Fig. 2. Time course of petiole break strength during exposure to C_2H_4. Explants from plants infected with vectors containing fragments of genes encoding Lc alone (*Lc*), or Lc with tomato abscission-related PG (*TAPG1*) were exposed to 1 μl l^{-1} C_2H_4, and the force required to separate the petioles was measured at the indicated times after the start of C_2H_4 treatment. Means of five measurements ±SD.

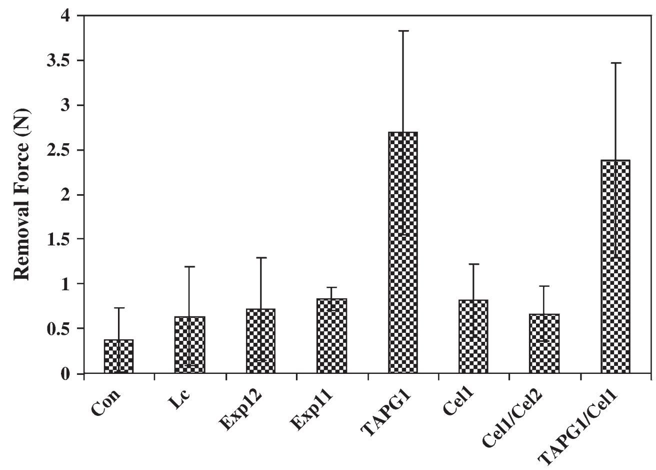

Fig. 3. Effect of silencing a range of cell wall-related genes on petiole abscission. Explants from control (Lc) plants, and plants infected with TRV vectors containing fragments of genes encoding Lc (Lc) alone, or combined with fragments of different expansins (*LeEXP11* and *12*), endoglucanase (*LeCEL1*), PG (*TAPG1*), two endoglucanases (*LeCEL1*/*LeCEL2*), or an endoglucanase and PG (*TAPG1*/*LeCEL1*) were exposed to 1 μl l^{-1} C_2H_4. The force required to remove the petioles from the axis was measured 60 h after the start of the C_2H_4 treatment. Means of at least five measurements ±SD.

that silencing *LeCEL1* had no effect on leaf abscission in tomato. This finding could be attributed to functional redundancy, with the activity of other endoglucanases compensating for the silencing of *LeCEL1*. However, even when *LeCEL1* and *LeCEL2* were simultaneously silenced, petiole break strength was unaffected (Fig. 3). Although the extent of silencing of these genes (given the limited quantity of tissue and therefore mRNA available) was not quantified, it has been shown in this study and others that the TRV/VIGS system results in very strong silencing of a range of genes (Table 4; Chen *et al.*, 2004, 2005; Xu *et al.*, 2007). Further study of the role of endoglucanases in petiole abscission will require silencing of multiple endoglucanases, if possible using silencing fragments that have high homology across the gene family. Even strong silencing of these genes might be insufficient to prevent translation of enough active protein, and complete silencing by a mutation approach would be required to conclusively demonstrate that they are not primary players in leaf abscission.

The result of silencing tomato PGs using a *TAPG1* fragment in the silencing vector was a significant delay in abscission and increased break strength of the abscission zone. Gonzales-Carranza *et al.* (2007) demonstrated that disruption of an abscission-related PG in *Arabidopsis* using a T-DNA insertion mutant delayed floral abscission. The fragment selected for the silencing construct used in this study has very high homology across all known tomato abscission-related PGs (Table 5). In addition, a highly homologous region (different from the silencing fragment) was used to measure transcript abundance. The primers used were completely homologous in the forward direction, and had only one mis-match for each sequence

Table 5. *Nucleotide sequence similarity among polygalacturonase genes in the target region*

	TAPG1	*TAPG2*	*TPG3*	*TAPG4*	*TAPG5*
TAPG1	100				
TAPG2	95.5	100			
TPG3	95.7	92.4	100		
TAPG4	86.4	85.4	86.1	100	
TAPG5	86.9	85.1	85.4	91.2	100

in the reverse direction. The expression data therefore are likely to indicate total *TAPG* transcript abundance, and the silencing system probably down-regulated more than one *TAPG* (Table 4). Since abscission still occurred in these silenced plants, it is clear that there are other actors in the cell wall dissolution process. It will be interesting to investigate the effects of silencing other cell-wall enzymes, alone and in combination. In particular, based on what is known about interactions between modifying proteins in fruit (Brummell *et al*., 1999*b*), it would be interesting to examine the effects on abscission of silencing expansins and PGs. The ease with which multiple genes can be silenced is one of the most attractive practical features of the VIGS system.

Tomato plants showed diverse phenotypes in response to silencing of PDS (Table 2), indicating the need to carefully select cultivars with adequate responses for silencing studies. Many cultivars showed 100% infection, and moderate-sized sectors of photobleaching in leaves, and some, such as 'Bradley Pink', combined high infection efficiency with large symptom sectors. Others, such as 'Pearson' showed relatively slight symptoms, although infection efficiency was high. The cultivar 'New Yorker', selected for this study, showed reliable silencing at high frequency, with moderately large sectors of photobleaching on the silenced leaves. Success in silencing *PDS* and other genes has been reported by two other research teams (Fu *et al*., 2005, 2006; Orzaez *et al*., 2006). The conditions for infection and growth of silenced plants were standardized, avoiding excessive damage to the delicate seedlings, and growing the infected plants at 22 °C, the optimal temperature for silencing (Table 3). We were interested in the relatively sharp temperature optimum for silencing, which suggests that movement and/or replication of the virus is strongly affected by relatively small temperature changes. In a recent study, Fu *et al.* (2006) reported that the silencing of PDS in the cultivar 'Lichun' was enhanced at low temperatures (15 °C) and low humidity, but their data show that this is more a matter of *when* the silencing is observed. Their data are in agreement with ours, in that young seedlings (2 weeks old) show optimum silencing at 21 °C and high humidity.

Although silencing PDS gives a clear phenotype, and was useful for developing optimal conditions for the VIGS system, the physiological and morphological effects of silencing PDS make it unsuitable as a silencing reporter. In petunia, the endogenous CHS was used as the reporter for silencing (Chen *et al*., 2004). In tomato, advantage was taken of an engineered line that was available, where anthocyanin expression was under the control of *Lc*, an activator gene cloned from maize that controls the expression of genes involved in anthocyanin biosynthesis (Goldsbrough *et al*., 1996). It was found that silencing CHS did not result in as clear a phenotype as that obtained by silencing *Lc*, so our standard system was to use a vector containing a fragment of *Lc* in tandem with fragments of target genes. Silencing resulted in substantially reduced anthocyanin content of the leaves and petioles (Fig. 1), and enabled tissues to be selected where our target genes would be silenced. This system has obvious potential for use in a wide range of studies of the function of genes in the growth and development of tomatoes.

Acknowledgements

We appreciate the assistance of Dr John Yoder, University of California Davis, in providing the seeds of transgenic purple tomato expressing *Lc*. We are grateful to the Tomato Genetics Resource Center (TGRC) at University of California Davis for providing tomato seeds of the other selections. This research was supported by Research Grant No. IS-3815-05 from BARD (the United States – Israel Binational Agricultural Research and Development Fund). We thank Drs Wouter van Doorn and Andrew Macnish for thoughtful comments on the manuscript.

References

Belfield EJ, Ruperti B, Roberts JA, McQueen-Mason S. 2005. Changes in expansin activity and gene expression during ethylene-promoted leaflet abscission in *Sambucus nigra*. *Journal of Experimental Botany* **56,** 817–823.

Brummell DA, Hall BD, Bennett AB. 1999*a*. Antisense suppression of tomato endo-1,4-beta-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Molecular Biology* **40,** 615–622.

Brummell DA, Harpster MH, Civello PM, Bennett AB, Dunsmuir P. 1999*b*. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *The Plant Cell* **112,** 2203–2216.

Campbell P, Braam J. 1999. Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying fuctions. *Trends in Plant Science* **4,** 361–366.

Chen J-C, Jiang C-Z, Gookin T, Hunter D, Clark D, Reid M. 2004. Chalcone synthase as a receptor in virus-induced gene silencing studies of flower senescence. *Plant Molecular Biology* **55,** 521–530.

Chen J-C, Jiang C-Z, Reid M. 2005. Silencing a prohibitin alters plant development and senescence. *The Plant Journal* **44,** 16–24.

Cho H-T, Cosgrove DJ. 2000. Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **97,** 9783–9788.

Clements J, Atkins C. 2001. Characterization of a non-abscission mutant in *Lupinus angustifolius* L.: physiological aspects. *Annals of Botany* **88,** 629–635.

del Campillo E, Bennett A. 1996. Pedicel break strength and cellulase gene expression during tomato flower abscission. *Plant Physiology* **111,** 813–820.

Fu DQ, Zhu BZ, Zhu HL, Jiang WB, Luo YB. 2005. Virus-induced gene silencing in tomato fruit. *The Plant Journal* **43,** 299–308.

Fu DQ, Zhu BZ, Zhu HL, Zhang HX, Xie YH, Jiang WB, Zhao XD, Luo YB. 2006. Enhancemant of virus-induced gene silencing in tomato by low temperature and low humidity. *Molecules and Cells* **21,** 163–160.

Goldsbrough A, Tong Y, Yoder J. 1996. Lc as a non-destructive visual reporter and transposition excision marker gene for tomato. *The Plant Cell* **9,** 927–933.

Gonzalez-Carranza ZH, Whitelaw CA, Swarup R, Roberts JA. 2002. Temporal and spatial expression of a polygalacturonase during leaf and flower abscission in oilseed rape and Arabidopsis. *Plant Physiology* **128,** 534–543.

Gonzalez-Carranza ZH, Elliott KA, Roberts JA. 2007. Expression of polygalacturonases and evidence to support their role during cell separation processes in *Arabidopsis thaliana*. *Journal of Experimental Botany* **58,** 3719–3730.

Hong SB, Sexton R, Tucker ML. 2000. Analysis of gene promoters for two tomato polygalacturonases expressed in abscission zones and the stigma. *Plant Physiology* **123,** 869–881.

Kalaitzis P, Koehler S, Tucker M. 1995. Cloning of a tomato polygalacturonase expressed in abscission. *Plant Molecular Biology* **28,** 647–656.

Kalaitzis P, Solomos T, Tucker ML. 1997. Three different polygalacturonases are expressed in tomato leaf and flower abscission, each with a different temporal expression pattern. *Plant Physiology* **113,** 1303–1308.

Lashbrook CC, Tieman DM, Klee HJ. 1998. Differential regulation of the tomato ETR gene family throughout plant development. *The Plant Journal* **15,** 243–252.

Liu Y, Schiff M, Dinesh-Kumar SP. 2002. Virus-induced gene silencing in tomato. *The Plant Journal* **31,** 777–786.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25,** 402–408.

Mishra A, Khare S, Trivedi PK, Nath P. 2008. Ethylene induced cotton leaf abscission is associated with higher expression of cellulase (GhCel1) and increased activities of ethylene biosynthesis enzymes in abscission zone. *Plant Physiology and Biochemistry* **46,** 54–63.

Orzaez D, Mirabel S, Wieland WH, Granell A. 2006. Agroinjection of tomato fruits: a tool for rapid functional analysis of transgenes directly in fruit. *Plant Physiology* **140,** 3–11.

Roberts JA, Elliott KA, Gonzalez-Carranza ZH. 2002. Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology* **53,** 131–158.

Tucker ML, Baird SL, Sexton R. 1991. Bean leaf abscission: tissue-specific accumulation of a cellulase mRNA. *Planta* **186,** 52–57.

Tucker ML, Milligan SB. 1991. Sequence analysis and comparison of avocado fruit and bean abscission cellulases. *Plant Physiology* **199,** 928–933.

Xu X, Jiang C-Z, Donnelly L, Reid MS. 2007. Functional analysis of a RING domain ankyrin repeat protein that is highly expressed during flower senescence. *Journal of Experimental Botany* **58,** 3623–3630.